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A BACTERIAL STEM BLIGHT OF
FIELD AND GARDEN PEAS

BY
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A BACTERIAL STEM BLIGHT OF FIELD AND GARDEN PEAS

By WALTER G. SACKETT

INTRODUCTION

Before the introduction of alfalfa into the San Luis Valley, field peas were grown very extensively as a leguminous crop for feeding purposes. Aside from their value in this role, their use in a crop rotation as a means of restoring partially the soil fertility lost by excessive and continuous cropping with small grains, was soon recognized.

For a number of years peas were used almost exclusively as the forage crop, and the acreage planted was very considerable; however, some five or six years ago, it was found that alfalfa could be grown successfully nearly everywhere in the valley. It succeeded very well for the first two or three years, but in 1914, following a heavy, late spring frost, the crop was attacked by the bacterial stem blight which reduced the tonnage of the first cutting 60 to 80 percent, to say nothing of the injury resulting to the plants themselves. Since then there has been a recurrence of the disease each year with varying degrees of severity. Quite naturally, with the appearance of this new trouble, the feeders may have to turn their attention more largely to field peas, but let us hope not to the exclusion of alfalfa.

Practically all of the cultivated land in the San Luis Valley lies at an elevation of between 7,500 and 8,500 feet, and at this altitude the temperature usually remains cool until well into the summer; frosts may be expected any month of the year. The soil is a sandy loam for the most part, and there is an abundance of irrigating water.

The pea being a lover of cool soil and cool weather, it is difficult to conceive of a location where conditions are better adapted to pea growing than in this old lake basin. For years they have been raised here to perfection without the first complaint of anything in the way of a bacterial, fungous or animal pest. But it is a long road that has no turning, for in the spring of 1915 the peas over almost the entire valley, which includes some 500,000 acres, were attacked by a disease, previously unknown in the community, and which at the time threatened to be even more serious than the alfalfa blight.

At about the same time that complaints were received of this trouble on field peas, a similar blight made its appearance on garden peas in Northern Colorado.

In the pages which follow, will be found a description of this disease and of the microorganism which has been found to be responsible for the trouble.

HISTORY AND DISTRIBUTION

The first occurrence of the disease was noted by Mr. E. H. Thomas, county agent for the San Luis Valley, on May 27, 1915, in the garden of Mr. Wm. Ollinger, Hooper, Colorado. In this instance, garden peas for family use had been planted in soil which had been very heavily manured, and when the vines were 3 to 6 inches high, they began to turn brown, dry up and die. The owner attributed the trouble to too much manure or to excessive alkali. However, Mr. Thomas was inclined to look upon these facts as of secondary importance, and accordingly sent the writer several specimens of affected peas at this time.

The general appearance of the plants, with their characteristic bruised, water-soaked leaves and stems, suggested, at first sight, bacteria as a very probable cause of the trouble, and subsequent experiments have confirmed this supposition.

The next locality where the disease was observed was in Blanca, where, on June 1, 1915, Mr. Thomas found the same trouble attacking field peas; during the next few days he noted the blight in the vicinity of San Acacio, Antonito, Monte Vista and Center. In fact, we are reasonably safe in saying that it was general on the early planted peas over practically the entire valley.

The writer visited the localities mentioned above on June 3 and 4. The peas varied considerably in size, ranging from 3 inches in height to 8 or 10, and in the two days that we spent in looking over the fields, we did not find more than two or three tracts that were entirely free from the trouble. The severity of the attack appeared to differ in the different localities, due possibly to the fact that the infection had progressed more rapidly and was farther advanced in some places than in others; now and then we came across a field where only an occasional plant was affected, but again there were many where it was almost impossible to find a healthy one.

Mr. Thomas visited one of the worst affected fields at Antonito on July 22nd. From the roadside, there appeared to be a full stand of good vines, but upon closer examination it was found that at least one-third of the plants were missing. The average branching of the remaining plants was heavier than normal, there being many with three or more vines, while under ordinary conditions only two would have been present. The old infection had dis-

appeared with the death of the first shoots, and the present crop was largely new growth. The vines were heavily podded and thrifty.

Mr. Thomas also visited a number of fields in the vicinity of Blanca and San Acacio on July 21, 1915. On sandy soil where the supply of moisture was limited, the peas had suffered heavily from blight. In two fields in particular, where the crop was planted on new ground, the disease destroyed fully three-fourths of the plants, but in the same locality on practically the same kind of soil where plenty of moisture was present, especially where an irrigation had been given shortly after the blight appeared, a large majority of the plants either threw out new branches, or, where they were not too badly infected, continued their growth.

The first isolations of the causal organism were made from material collected at Antonito on June 4, 1915.

VARIETIES OF FIELD PEAS

The two varieties of field peas that are planted most extensively in the San Luis Valley are the native White Mexican and the Warshauer.

The Mexican is probably a mixture of Canadian Beauty and Golden Vine with French Grey and possibly a few Early Britain.

The Warshauer is a white pea that has been developed by the Warshauer-McClure Sheep Company, of Antonito, Colorado, by hand picking from peas that were two weeks earlier than the common Mexican. It is supposed that the seed originally planted, when this selection was made, was one of the garden sorts usually considered as, "Earliest and Best". However, the pea has been reared under field conditions and continually hand picked until the Warshauer pea is now a decidedly fixed type. It is a medium-vined early pea which begins to blossom when 6 to 8 inches high, resulting in a good distribution of pods thruout the entire length of the vine.

On June 15, 1915, we received a letter from the Empson Packing Company, of Longmont, Colorado, stating that they were sending us some pea vines which appeared to be attacked by a blight. They said further that nothing of the kind had ever occurred before in the history of their canning business, and that altho the first traces had been noted only three or four days previously, the trouble was spreading rapidly. Already a number of fields were affected, and no little alarm was expressed over the possible outcome.

This outbreak of the disease on garden peas, contemporaneous with that on field peas in an entirely different part of the State,

more than 300 miles distant and at an altitude 2,500 feet lower, was of more than passing interest. This led the writer to visit the Longmont section on June 21, in company with Mr. H. J. Cains, of the Empson Company. We found the garden peas affected in the same identical manner as the field peas in the San Luis Valley.

One field of about 12 acres was in very bad condition. The attack was well advanced and general; the vines were watery, olive-green to black in color; many were entirely destroyed, their growing tips curled and dead; others were green and in blossom, while some bore small pods. It was practically impossible to find a perfectly healthy plant in the whole 12 acres. The crop of shelled peas from this field was estimated at 50 percent. This same land was in peas the preceding year, but no disease was observed.

The blight was present, but to a lesser degree, in an adjoining tract of approximately the same size. In both cases the Alaska pea had been planted, the sowings having been made between the 15th and 20th of April.

A few infected plants were found in each of two other fields of the same variety of a somewhat later sowing.

A careful examination was made of several other Alaska plantings which were sown from ten days to two weeks later than any of the above, but in no instance did we find any of the trouble.

In addition to the Alaska, which is the earliest pea planted by the Empson Packing Company, two later sorts are grown for canning purposes. A diligent search for the disease on these later varieties failed to show even a trace.

A number of gardens in Fort Collins experienced a mild attack of blight on the early peas, but in no case was it sufficiently serious to cause any appreciable loss to the crop.

From these observations of one season only, it appears that so far as garden peas are concerned, only the earliest plantings of the early varieties are affected, and that the later sorts are practically free from the trouble.

We have endeavored to ascertain information regarding the distribution of the disease in the Western States, from the respective experiment stations, and up to the present time it has not been observed in Kansas, Montana, Wyoming, Nevada, Idaho, Arizona, or New Mexico. Professor J. J. Thornbee, of the Arizona Station, recalls having seen such a disease in both Nebraska and South Dakota. Professor G. R. Hill, of the Utah Station, reports the trouble as occurring in 1914 at Riverdale, Utah. Professor F. D. Heald, of the Washington Station, has recorded a bacteriosis of peas which may be the same as the Colorado malady. He has very kindly submitted pressed specimens for our examina-

tion, and while it is difficult to reach any definite conclusions from dried material, I am inclined to believe that the Washington disease is different from that which occurs locally.

DESCRIPTION OF THE DISEASE

The disease can be recognized very readily by the watery, olive-green to olive-brown color of the stems, and by the yellowish, bruised and watery appearing stipules and leaflets.

When the blight makes its appearance early in the season, before the plants are more than 8 to 10 centimeters high (3 to 4 inches), one usually finds the stand very uneven and scattering. It is not uncommon to see 4 to 6 feet of drill-row apparently without a single plant, but a closer examination of the ground will reveal the brown and withered remains of what were once small pea vines. Here and there may be located a few stragglers that have not yet succumbed; they usually have several dead leaves next to the ground, while above these are four or five that appear bruised, watery and often ochre-yellow in color. (Right-hand figure in colored plate.) This leaf description applies particularly to the large, clasping stipules, the leaf-like structures at the base of the leaf stalk. (Left-hand figure in colored plate.) The stems of such plants are almost always dark-brown or black and considerably shrivelled, and so far as any further growth is concerned, they are practically dead. Occasionally one meets with a young plant of this kind in which the stem is black next to the ground, but above which both the stem and leaves are normal in color and look perfectly healthy.

In older plants, say 12 to 15 centimeters high, (5 to 6 inches) where infection appears to have taken place at a later period, the stem is watery, olive-green to olive-brown in color (middle figure colored plate); the petioles of the leaves attached to the diseased portion soon become involved, turn watery, olive-green in color, seem to wilt or collapse and allow the leaves to droop; the leaf blades and stipules subsequently appear bruised and watery along the veins and mid-rib, which condition is communicated to the leaf tissue proper, and the whole leaf structure turns a watery, ochre-yellow color, wilts and dies, shrivelled and brown.

So far as our present knowledge goes, the root and that part of the stem which is below ground are not affected.

The infection starts in most cases from 2.5 to 3 centimeters (1 to 1.25 inches) above the ground line and seems to move up the stem toward the growing tip. This is evident from the facts both that the discoloration on the lower part of the vine is nearly always darker than that higher up, indicating an older lesion, and that the lower leaves are the first to die.

Under ordinary field conditions, a single pea plant branches at its base to form two or three principal vines. If the blight attacks and destroys one or more of these, the plant will usually throw out from three to five new ones, from the region below the diseased area, providing sufficient vitality remains to promote this growth. As a matter of fact, this is usually what takes place in the field, and, as a rule, the new growth progresses unmolested, altho, occasionally, this too is attacked. The secondary shoots are often small, and do not make the rank and vigorous growth that is characteristic of the earlier ones.

If the vines become diseased when they are small, and the main vine dies before branching has taken place, the chances are that no branches will be formed, and the plant will be killed outright. Sometimes even the older plants behave in this manner.

The leaves may develop the disease independently of any other structure and die before the stems show any trace of the trouble.

Very rarely small droplets of a pale yellow exudate are found on the stems.

The close resemblance in the appearance of alfalfa plants affected with the bacterial stem blight and that of diseased pea vines is a matter of considerable interest. In fact, the symptoms of the disease in the two plants are so similar as to justify one in making the conjecture that it is all one malady, and that one and the same causal agent is responsible for this condition. The pea and the alfalfa, both being legumes, and both grown in the same soil, under the same climatic conditions, and in a locality where the alfalfa blight has been very severe, the natural inference would be that both plants were suffering from the same cause. This is not the case, however, since the organism which we have isolated from the diseased pea vines and which we have been able to show is the specific cause of the pea trouble, is a separate and distinct form differing both morphologically and culturally from *Ps. medicaginis*, the alfalfa blight organism.

If fragments of the watery, olive-green tissue from either stem, leaf or petiole are mounted in a drop of water on a glass slide, there soon appears about the specimen a milky cloud easily visible to the naked eye. Under the low power of the microscope this resolves itself into a finely granular mass which flows out in all directions in long streamers, so characteristic of infections of this sort. Under the high power, the microorganisms which make up the milky cloud are readily distinguishable as moderately motile rods from two to four times as long as broad.

HISTOLOGY OF THE DISEASED TISSUE

Celloidin sections of diseased stems, killed in absolute alcohol, show the substomatal chambers to be packed with bacteria and

many of the surrounding cells of the underlying parenchyma to be filled likewise. (Fig. I.) The infection appears not to extend into the medullar parenchyma, stereome, or vascular bundles. Neither in the field nor when artificially inoculated in the greenhouse, have we observed the vines, as a whole, to wilt; however it is not uncommon to see the petioles and leaflets in this condition. Frequently plants that have been inoculated artificially show a gradual wilting, or perhaps better designated as withering, in two or three weeks after infection, but not the sudden and complete

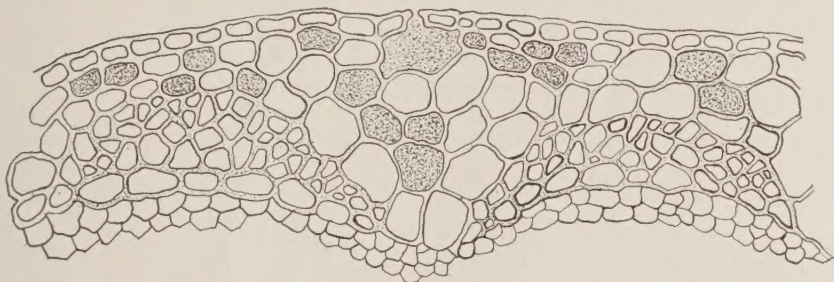


FIG. I

prostration of the entire structure such as is usually meant by the term *wilting* in the technical sense. Owing to the lack of satisfactory histological material, we are unable to say in the latter case whether or not there has been an actual invasion of the vascular system.

ISOLATION OF THE CAUSAL ORGANISM

The material from which the first isolations were made was collected at Antonito, Colorado, June 4, 1915. None of the tissue was given any preliminary sterilization previous to plating, but care was exercised not to use any that was clearly and unquestionably contaminated by soil or otherwise. Dilution plates were made June 5th in +10 nutrient agar, three plates being used in each case. These were incubated for 60 hours at 28° C., at the end of which time colonies were plainly visible.

ORIGINAL ISOLATIONS FROM FIELD MATERIAL, JUNE 5, 1915

No. 1. Stem.—A recent infection; tissue bright and fresh appearing, but watery and discolored well up on the stem; thin transverse section used for plating taken from near growing tip where disease was just developing. Colonies appeared in 60 hours; grayish white by reflected light, bluish cast by transmitted light.

glistening, raised, structure appears flocculent under hand lens, gyrose under low power, margin undulating, general outline of colony round. Plates pure. Two colonies picked up on agar slants.

No. 2. Petiole from Stem No. 1.—Petiole taken from part of stem where disease was advanced, tissue dark and watery; section 0.5 mm. long, near juncture with stem, removed for plating. Colonies visible in 60 hours; same as those obtained from No. 1, but first two plates contaminated, third plate pure. Made subcultures from two typical colonies.

No. 3. Petiole from Stem No. 1.—Petiole taken from part of stem where disease was just appearing; tissue watery and olive-green in color. Colonies appeared in 60 hours; same as obtained from No. 1; culture pure in second and third plates. Made cultures from two typical colonies on nutrient agar.

No. 4. Leaf Blade from Petiole No. 3.—Material used in plating taken from dark, watery part of leaf blade next to midrib; leaf well up on plant and clean. Colonies appeared in 60 hours; same as preceding; pure in second and third plates. Picked up two characteristic colonies.

No. 5. Stem.—Recent infection; tissue light olive-green and watery. Colonies appeared in 60 hours; same as in preceding isolations; plates pure. Picked up two typical colonies on agar slants.

INOCULATION AND REISOLATION EXPERIMENTS

PEAS

September 21, 1915.—Four varieties of peas were used at this time: Warshauer, Horsford, Alaska and Wellington. The plants were grown in 5-inch pots, one variety to a pot, and were fourteen days old when inoculated.

A 24-hour agar culture of the organism that was isolated from Stem No. 1 was used in this series; the agar growth was worked up in the water of condensation at the bottom of the slant, and the suspension thus obtained was employed as the inoculum. The stems to be inoculated were first scarified lightly with a sterilized scalpel until the surface was slightly moist, and then several loopfuls of the culture were spread over the prepared spot, 1 to 2 centimeters in length. Following this, the culture was pricked into the tissue with a sterilized needle. Three to four scarifications, 2 to 3 centimeters apart, were usually made on each plant, and one plant thus treated was left in each pot uninoculated for a check. They were sprayed immediately after infection with sterile, distilled

water from an atomizer, and placed under bell jars in subdued north light in the laboratory. The bell jars were removed after 48 hours, but spraying was continued for three days more. On September 27, the plants were taken out-of-doors and left in bright sunshine. The inoculated areas on all plants appeared watery and olive-green in color, as compared with the check four days after infection. By the end of six days, there was little change, except for a suggestion of a watery zone beyond the edge of the scarified areas; the Wellington peas remained unchanged.

When observed on October 5, the watery zone had spread about 4 mm. beyond the scarifications, in all except the Wellington, and tissue removed from this part of the stem swarmed with bacteria when examined under the microscope.

By October 7, all varieties, except the Wellington, had developed the disease without question. It had now spread to the stipules, giving them a watery appearance, and in some cases, had produced a yellowing along the veins just as under field conditions. One leaf, that had been inoculated by a needle prick, had developed a large yellow blotch, such as occur in the field. The yellow, olive-green color and watery character had spread along the stem some distance from the original scar. We had had two hard frosts on the 5th and 7th of October, and the plants had been exposed to both.

October 12, 1916.—After two more heavy frosts, all of the plants were taken up, and a careful examination made. One of the most interesting points brought out in the experiment was the apparent lowering of frost resistance. Without exception, all of the plants in which the disease had made any progress succumbed to the frost and wilted, while every check and all of the Wellington plants stood up bright and fresh and showed no effects of the cold whatever. It would appear from this that the Wellington variety is more nearly immune than any of the others.

Observations October 12, 1915

Warshauer plants.—Check still green. Three plants were inoculated, and all show typical olive-green to brown discoloration. This is not confined to the inoculated areas, but has spread over the whole stem; vines somewhat shrivelled and tissue swarming with bacteria.

Horsford plants.—Check in good condition, still green and little affected by frost. Four plants were inoculated; before frost, the watery, olive-green color had spread over the stems irrespective of the original infection; after frost, the vines are shrivelled and brown, and bacteria are very abundant in the tissue.

Alaska plants.—Check normal. Three plants were inoculated; one has developed the disease in an unbroken lesion 12 centimeters

long from the ground up; the others show the trouble just in the immediate region of the scar. In two of them, the infection has spread from the stem into the stipules, giving them a watery appearance which has become yellowish brown in the older lesions. The frost has shrivelled all the vines except the check. Bacteria abundant in affected tissue.

Wellington plants.—Four plants were inoculated with fourteen scarifications in all. As mentioned above, the frost seems not to have injured them appreciably. Almost no discoloration of the stems has occurred beyond the edge of the scarifications, and the vitality and general appearance of the plants are good. A little shrivelling has taken place where the inoculation was made, but there is no indication that the infection has spread. Bacteria are still present at the edges of the old lesions where the tissue appears darker and more watery, but none can be found in the scar proper. Apparently immune.

INOCULATIONS OF OCTOBER 19, 1915.—PEAS

The plants of this series were inoculated by pricking (cluster of stabs) the culture into the stem with a sterilized needle without first scarifying the surface. A 24-hour agar culture of the organism isolated from Stem No. 1 was used. All plants were sprayed with sterile distilled water for four days after inoculation, and kept covered with bell jars for three days.

Observations

Warshauer.—Five plants inoculated, and one check. After four days stems begin to have watery appearance at point of infection; check normal in color. After twenty days, stems dark olive-brown and shrunk, leaves withered and brown, practically dead; check normal.

Horsford.—Four plants inoculated midway of the vine toward the growing tip; one uninoculated check. After four days, sunken watery areas appear around the needle pricks; at the end of eight days, two of the plants are so badly shrunk and collapsed at points of inoculation as to allow the whole of the tips to droop. A microscopic examination of the watery tissue taken 1 cm. from needle prick shows the cells to be gorged with motile bacteria. The two remaining plants are watery and discolored, but have not collapsed. Check shows nothing of this shrivelling or discoloration. Causal organism reisolated from one of the two worst affected plants, q. v. page 18.

INOCULATIONS OF NOVEMBER 25, 1915.—HORSFORD PEAS

All of the inoculations heretofore were made with one of the cultures isolated from Stem No. 1 in June. In order to determine

whether the remaining nine cultures, which were isolated at the same time, were likewise responsible for the disease and still virulent, each of nine Horsford plants was inoculated with a 48-hour agar culture of the respective organisms, to-wit: One from stem (No. 1), two from petiole (No. 2), two from petiole (No. 3), two from leaf blade (No. 4), two from stem (No. 5).

In this case, the bacteria were introduced into the stems by single shallow needle pricks about 1 centimeter apart and were distributed over the younger part of the vine. In addition to this, several leaves on each plant were punctured and the culture smeared over the injured leaf surface. All plants were sprayed with sterile, distilled water immediately after inoculation, covered with bell jars and placed in subdued light. They were sprayed again after 36 hours, and after 48 hours they were uncovered and placed in the greenhouse.

Observations

After four days, each of the inoculated plants shows a decided watery zone around each needle prick in the stem, and all of the leaf infections except the one made with culture from Stem No. 5 are taking perfectly; the leaves appear watery and bruised not only around the needle puncture, but also in isolated spots where the culture was rubbed over the surface; this suggests stomatal infection. The disease seems to be progressing most rapidly in the plant that was inoculated with one of the cultures isolated from the leaf blade. (No. 4.)

Dec. 1, 1915.—The watery, bruised appearance of the leaves is spreading and in some cases the leaves are beginning to curl.

Dec. 3, 1915.—The stem pricks now show up as sunken yellow areas, 3 mm. long.

Dec. 6, 1915.—Stems now dark olive-green and watery, not only around the needle pricks, but continuous over the entire stem where inoculated.

Dec. 7, 1915.—Reisolation made from plant inoculated with culture isolated from leaf blade q. v. page 18.

March 31, 1916.—All inoculated shoots brown and dead; four plants are entirely destroyed and have thrown out no new growth from below; one plant has two new shoots, while the remaining three have one each; none of these is vigorous, due possibly to other causes. Check normal.

INOCULATIONS OF DEC. 17, 1915.—HORSFORD PEAS

Nine different cultures, presumably all the same organism, picked up from colonies which were obtained by plating out tissue from a plant infected in the laboratory November 25 with a pure

culture from a diseased leaf blade, were used in this experiment. Each of nine pea vines, approximately 15 centimeters in height, was inoculated with one of these cultures; the germs were introduced by needle pricks both in the stems and in the leaves. The plants were kept covered with bell jars for 48 hours and sprayed with sterile distilled water at frequent intervals; at the end of this time they were placed in the greenhouse.

Observations

Dec. 21, 1915.—All of the inoculations on the stems seem to have taken nicely, as they show watery, slightly sunken areas around the needle pricks.

Dec. 22, 1915.—The watery zone around the needle punctures more pronounced than the day before; many of the leaves are beginning to show watery spots.

Jan. 3, 1916.—While we are obtaining positive results unquestionably with all nine cultures, two of them seem to be somewhat more virulent than the others, as the disease is progressing more rapidly in the two plants inoculated with these particular strains. The watery spots encircling the needle punctures have coalesced into continuous dark olive-green lesions extending over the inoculated portion of the stems. All appear practically the same, but the area involved is considerably greater in the two cases mentioned above. Some of the leaves are shrivelled, others show watery spots and irregular yellow blotches; growth seems to be checked.

Jan. 31, 1916.—The inoculated shoots on all nine plants are dead and black; three of the plants have thrown out a single new shoot from below and three have responded with two new shoots; three died without producing any new growth at all. Fig. II is a photograph, taken at this time, of the two plants with the two new shoots; note in each case the dried, inoculated, dead vine between the two healthy ones. A third check plant is in the same pot.

INOCULATIONS OF JANUARY 6, 1916.—HORSFORD PEAS

The two cultures which appeared to be the most virulent in the preceding series, and which produced the most typical symptoms of disease in the host were selected for reinoculation in the present experiment. For convenience, these may be designated as Numbers 3 and 8.

Four plants, 7 cm. in height, were inoculated with Culture 3 by means of single needle pricks in a line along the stem. Three similar plants were likewise inoculated with Culture 8; 72-hour agar cultures were used in both cases. One check plant was left



FIG. 11

in the No. 3 pot. All plants were sprayed with sterile, distilled water, placed in greenhouse under bell jars and protected from bright sunlight by newspapers.

Observations

Jan. 7, 1916.—Sprayed plants; no indication of disease yet.

Jan. 10, 1916.—Removed bell jars and newspapers. All plants, except check, show watery, sunken zones around needle pricks.

Jan. 13, 1916.—The separate watery areas have now coalesced so that the stems exhibit a watery, bruised aspect over the entire section that was inoculated. Both cultures seem to be working equally well. Check still unchanged.

Jan. 24, 1916.—Three of the plants inoculated with Culture 3 are turning yellow and the tips of two are wilting, due apparently to collapsing and girdling of the stems, which are now dark

olive-brown in color thruout their entire length; the fourth plant is reacting positively, but slowly.

Two of the plants inoculated with Culture 8 are still erect, but one is turning yellow and wilting, as described above; the stems of all the infected shoots are dark olive-brown in color.

Feb. 19, 1916. Culture 8.—One plant dead; one plant with inoculated shoot dead and two new shoots; one plant with inoculated shoot dark olive-brown and two new shoots.

Culture 3.—The inoculated shoots of all four plants dead. Three plants have two new shoots, and one has one new shoot.

INOCULATIONS OF FEBRUARY 19, 1916.—HORSFORD PEAS

Thirteen vigorously growing pea shoots about to blossom were inoculated with a 24-hour agar culture of Culture 3 by a line of single needle pricks; a fourth plant was pricked with a sterile needle as a control. The shoots were sprayed with sterile, distilled water and kept covered with a bell jar for 36 hours. When examined at the end of this time, watery zones were visible around each needle prick for more than 1 mm., while the check showed nothing but the hole left by the puncture.

Observations

After four days the watery areas surrounding adjacent punctures had coalesced to such an extent as to produce a solid infection over the whole stem. Control normal.

March 8, 1916.—One whole stem dark olive-green, and infection has spread over entire shoot; the disease has not spread as rapidly over second shoot, due possibly to the fact that the inoculation was in older tissue; third vine was accidentally broken off. Check normal.

March 31, 1916.—One plant, which was the less affected, has a black stem for 12 centimeters above ground, but it does not appear to be suffering particularly from the disease; it is growing, blossoming and seems vigorous. The inoculation in this case was on the lower part of the stem in rather old, hard tissue, and as has been observed repeatedly, infections in the older tissue do not have the same disastrous effects as when they are made in the younger. The second plant was inoculated a little higher up, and while in practically the same condition as the other, it is not as vigorous.

INOCULATIONS OF MARCH 9, 1916.—HORSFORD PEAS

To make certain that our stock culture of Culture 3 was remaining pure, it was replated March 4, 1916, and a new culture picked up from agar colony March 8.

In order to verify the new culture, it was inoculated from a 24-hour agar streak into two pea vines that were in blossom. One shoot was pricked with a sterile needle for a check. All plants were in the same pot. They were sprayed as usual and covered with bell jar and newspaper for 48 hours. Placed in greenhouse.

Observations

March 13, 1916.—All inoculations are taking; sunken watery areas, 3 mm. in diameter, around each needle prick, and where these are close together the lesions have coalesced, making a continuous infection the entire length of the stem.

March 31, 1916.—Inoculated shoots dark olive-brown, watery, and shrivelled; discoloration and watery appearance extends from stems along mid-ribs of stipules, causing a partial wilting of the latter. Dark, watery spots also present on leaves and stipules, which suggest stomatal infection probably resulting from washing germs from inoculated stems onto leaves while watering. Both infected vines have a sickly, pale-yellow color and are partially wilted. The check plant shows infection in two spots where the culture has apparently dripped during watering.

April 14, 1914.—All shoots brown and dead, including check, from lowest point of inoculation out to the growing tip; lower part of vines green.

INOCULATIONS OF MARCH 23, 1914

The purpose of this series was to determine whether the organism which is responsible for the pea disease is likewise pathogenic for other common legumes. Twenty-four hour agar cultures were used in all of the infections. In addition to needle prick inoculations in the stems and leaves, the entire plants were sprayed with a suspension of the culture in physiological salt solution. Just previous to this operation, they were thoroly sprayed with distilled water and then with the culture, until the liquid dripped from the leaves. Our object in spraying with the culture was to determine whether the stomata might not serve as a channel of infection, as well as mechanical injuries. All plants were sprayed with distilled water at frequent intervals and kept covered with bell jars for 48 hours after inoculations.

Horsford Garden Pea.—After four days, characteristic watery, olive-green depressions appeared around the needle pricks. After six days, splendid stomatal infections, appearing as dark, watery, green spots, developed on the leaves which had not been subjected to mechanical injury. (Plate II, Fig. 1.)

Golden Wax Bean.—After four days, there appeared around the needle pricks on the bean pods, grayish brown patches, .95 mm.

in diameter. Similar injury with a sterilized needle did not produce the same results. Inoculated stems and leaves were unchanged.

April 14, 1916.—The spots on the bean pod remained unchanged and there was no evidence of the disease in any other parts of the plants.

Alfalfa.—April 14, 1916.—The plants appeared to be entirely immune to the disease.

Sweet Clover.—April 14, 1916.—Entirely immune.

Crimson Clover.—April 14, 1916.—Entirely immune.

Mammoth Clover.—April 14, 1916.—Entirely immune.

Cow Pea.—April 14, 1916.—Entirely immune.

REISOLATION OF CAUSAL ORGANISMS FROM PLANTS INOCULATED WITH PURE CULTURES

First Reisolation, October 27, 1915

Platings in nutrient agar were made from sections taken from pea stems which had been inoculated October 19 with a pure culture from Stem No. 1 and which had developed the blight with all the typical symptoms. The tissue used came from two different parts of the vine where it appeared watery and olive-green in color some distance from the point of infection. No attempt was made to sterilize the surface of the stems from which this was taken, previous to plating. The plates gave a pure culture, and from the resulting colonies, eighteen were picked up on agar slants; ten of these were selected and reinoculated into Warshauer field peas November 15. Typical blight resulted in every case.

Second Reisolation, December 7, 1915

The plant from which the reisolation was made was inoculated November 25, 1915, with one of the cultures originally isolated from an infected leaf blade, June 4, 1915.

Two sets of plates were made from this plant, one from the stem and one from the base of a watery petiole where the disease was just starting. The stem was yellow, watery and sunken over the whole area of needle pricks, not only around them, but between, as well.

In all former isolations, no precautions were taken to sterilize the outside of the stems previous to plating out the tissue. However, in the present case it was wiped with alcoholic mercuric

chloride, 1-1000, after which a section to be plated was removed with a sterile scalpel. It was next transferred to alcoholic mercuric chloride (1-1000) for 15 seconds, then washed in three changes of sterile, distilled water. From the distilled water it was transferred to a tube of nutrient broth where it was crushed, and dilution plates were made from the suspension thus obtained.

The second set of plates was made directly from the watery petiole without any previous sterilization.

Pure cultures of the original organism were obtained from both sets of plates in the second and third dilutions; the colonies in the first dilution were so numerous, that it was impossible to tell anything about their purity.

Three colonies were picked up from the first set of plates and six from the second, making nine pure cultures in all, of presumably the same organism.

On December 17, these were all inoculated into pea plants, as described before on page 13 with positive results.

DESCRIPTION OF THE CAUSAL ORGANISM

Pseudomonas pisi, n. sp.

History.—The microorganism herein described as the cause of a bacterial stem blight of field and garden peas, and to which the writer has given the name *Pseudomonas pisi*, n. sp., was isolated June 4, 1915, from the watery portion of a diseased leaf blade. It was grown upon +10 nutrient agar until November 25, 1915, when it was inoculated into a pea plant, where it produced all of the symptoms characteristic of the disease. On December 7, 1915, a reisolation of the organism was accomplished, and after replating to insure purity, it was again inoculated into peas on December 17, 1915, January 6, 1916, February 19, 1916, March 9, 1916, and March 23, 1916. In every case, positive results have been obtained. The culture which has been used in the following species description came from the same stock as that used in the plant inoculations of December 17.

I. MORPHOLOGY

I. VEGETATIVE CELLS.—As the organism occurs both on culture media and in the plant, it is a short rod with rounded ends, mostly single and in pairs (Plate I, Fig. 1), but occasionally in fours and sixes; filaments have been obtained from very young agar cultures 10 to 97 μ in length. (Plate I, Fig. 2.) The individual rods measure 1.11 to 3.28 μ long and .58 to .82 μ wide. The length of the majority is 2.26 μ and the width, .68 μ .

2. ENDOSPORES.—No spores have been observed. Agar cultures 6 days and 48 days old were stained for spores both with Moller's stain and with Zeihl-Nielson carbol fuchsin, with negative results.

3. FLAGELLA.—The organism is motile by means of a single polar flagellum readily demonstrable in 18-hour agar cultures stained by Loeffler's method. According to Migula's classification, this places the organism in the genus, *Pseudomonas*.

4. CAPSULES.—No capsules could be demonstrated either by Welch's method or by that of Hiss.

5. ZOOGLOEA.—No zoogloea have been observed.

6. INVOLUTION FORMS.—None observed.

7. STAINING REACTIONS.—The germ stains readily with all of the ordinary aqueous stains. Both aqueous and carbol fuchsin bring out the coarsely granular protoplasm of the cell, together with what appear to be polar bodies. Loeffler's methylene blue shows nothing unusual altho at times there is a suggestion of irregular staining. Neisser's stain reveals neither polar bodies nor any special intracellular structures. It is Gram negative and is not acid fast. Neither starch nor glycogen were demonstrated. Both anilin-water genitan violet and anilin water fuchsin stain it readily.

II. CULTURAL FEATURES

1. AGAR STROKE.—*Growth* moderate in 24 hours; abundant in 4 days. *Form of growth*—filiform, margins irregular. *Elevation*—flat. *Lustre*—glistening. *Topography*—smooth. *Optical characters*—translucent. *Chromogenesis**—grayish white, Engine Color No. 13. *Odor*—decided, rotten cabbage. *Consistency*—butyrous. *Medium*—slightly greened.

2. POTATO.—*Growth*—moderate, persistent. *Form of growth*—filiform. *Elevation of growth*—slightly raised. *Topography*—smooth. *Odor*—absent. *Chromogenesis*—cream, orange yellow tint No. 2 in three days, Engine color No. 7 in five days (light orange). *Consistency*—waxy and slightly viscid. *Medium*—gray-brown in fourteen days.

3. LOEFFLER'S BLOOD SERUM.—*Stroke*—moderate in 72 hours. *Form*—filiform. *Elevation*—slightly convex. *Lustre*—glistening. *Topography*—smooth. *Chromogenesis*—pale yellow, Engine color 3a. No liquefaction. *Medium* unchanged.

*Color terms refer to Standard colored papers made by Milton Bradley Co., Springfield, Mass.

4. GLYCERINE AGAR.—*Growth*—abundant. *Form of growth*—filiform. *Elevation of growth*—slightly raised. *Lustre*—glistening. *Optical characters*—translucent. *Topography*—smooth. *Chromogenesis*—grayish white, Engine color No. 13. *Odor*—sour peas. *Consistency*—butyrous. *Medium*—greened.

5. MANNITE AGAR.—*Growth*—scant, just sufficient to define line of inoculation. *Form of growth*—filiform. *Elevation*—slightly raised. *Lustre*—glistening. *Topography*—smooth. *Optical characters*—translucent. *Chromogenesis*—color of boiled starch. *Odor*—absent. *Consistency*—butyrous.

6. GELATIN STAB.—*Growth*—best at top. *Line of puncture*—filiform. *Liquefaction*—begins in 24 hours; crateriform in 48 hours, broad funnel-shaped in three days (Plate II, Fig. 2), stratiform in seven days. (Plate II, Fig. 3.) Depth of liquefaction in tube 10 mm. in diameter, evenly inoculated, at the end of 30 days at 20° C. was 33.0 mm. Liquefaction in tubes regular size, 150x16 mm. complete after 102 days. *Color of medium* unchanged.

7. NUTRIENT BROTH.—*Surface growth*—scum in five days which becomes heavy, flocculent growth in ten days; sinks on agitation. *Clouding*—strong, persistent, fluid turbid. *Odor*—decided, putrefactive. *Sediment*—scant, compact and finely granular.

8. PLAIN MILK. 28° C.—*Coagulation* delayed until the fourth or fifth day, when a soft, more or less flocculent, curd is formed. *Extrusion of whey* begins in four days. *Coagulum* is slowly peptonized, the digested portion or liquid becoming light yellowish green in color. *Peptonization* begins on fourth day, 82% being digested in 21 days, and is complete on the 52nd. Reaction, 1 d. + 12, 2 d. + 12, 4 d. + 12, 10 d. + 4, 20 d. — 4. *Consistency*—soft curd. *Lab ferment* present.

20° C.—*Coagulation* delayed until the sixth or seventh day, when a soft curd is formed. *Extrusion of whey* begins in seven or eight days. *Coagulum* is slowly peptonized, the liquefied portion becoming pale lumuire green* in color. *Peptonization* begins on the eighth day, 60% of this curd being digested in 21 days and is not quite complete after 58 days. One-hundred c.c. portions in 200 c.c. Erlenmeyer flasks were completely peptonized in 28 days. Reaction 1 d. + 12, 2 d. + 12, 4 d. + 12, 10 d. + 4, 20 d. † *Consistency*—soft curd. *Lab ferment* present.

9. LITMUS MILK. 28° C.—Lavender-colored litmus milk begins to turn blue from the surface downward, becoming lighter

*Color Standards and Color Nomenclature—Ridgway.

†The development of by-products at the end of 20 days interfered so seriously with the determination of the end point that it was practically impossible to ascertain the reaction at this time.

below, and is two-thirds blued after ten days. Following this, the litmus is reduced and the whole tube assumes a gray color in fifteen days. At the end of twenty days the peptonization is complete and the fluid is dark blue to dark blue green in color. Coagulation begins on the fifth day, followed by clearing, which is complete in twenty days.

20° C.—Milk begins to turn blue from the surface downward after seven days, becoming lighter below, and is completely blued after fourteen days. Following this the fluid becomes gray with a blue ring at the surface, and at the end of 35 days it is between dark blue gray and dark green gray, *i. e.* green gray No. 2 and A-blue green dark, in color. Coagulation begins the seventh day, followed by peptonization, which is complete in 35 days.

10. GELATIN COLONIES.—*Growth*—moderately rapid at 20° C. *Form*—round. *Elevation*—crateriform, liquefying. *Edge*—entire. *Liquefaction*—saucer. *Size*—4 mm. diameter in three days.

11. AGAR COLONIES.—*Growth*—moderately rapid at 20° C. *Form*—round. (Plate I, Figs. 3, 4.) *Elevation*—slightly convex. *Edge*—undulate. *Internal structure*—gyrose, marked by wavy lines indefinitely placed. (Fig. III.) *Size of majority*—2 mm. in five days. Deep colonies lens-shaped. (Plate I, Fig. 5.)



FIG. III

12. STARCH JELLY.—Prepared from Uschinsky's solution and potato starch. *Growth*—moderate. *Medium*—stained yellowish green. *Diastatic action*—absent.

13. COHN'S SOLUTION.—No growth.

14. USCHINSKY'S SOLUTION.—No growth.

15. FERMI'S SOLUTION.—Growth abundant, heavy clouding, white wrinkled pellicle at surface in ten days and small amount of sediment.

16. NAEGLI'S SOLUTION.—Slight transient clouding.
17. FRAENKEL'S SOLUTION.—Heavy clouding and surface scum in ten days.
18. DUNHAM'S SOLUTION.—Uniform, heavy clouding and considerable white compact sediment, somewhat viscid on agitation.
19. ASPARAGIN SOLUTION.—Growth, abundant. Fluid, colored light green.
20. SOHNGEN'S SOLUTION.—(Urea.) No growth.

21. SODIUM CHLORIDE IN BOUILLON.—Nutrient broth containing 1% peptone, but without salt, was used in this determination. To measured quantities of this medium, different amounts of chemically pure sodium chloride were added, giving a range in the salt content from 0.0% to 5.0%. Four tubes of each concentration were prepared, and each set differed from the others by 0.25%. One loop of a 24-hour broth culture was used for the inoculation, and the tubes were kept at 28° C.

Growth occurred in 24 hours in all concentrations up to 2.0%. No difference could be observed in the amount of growth from 0.0% to 0.75%, however, with 1.0% and 1.25% there was less, and with 1.5% and 1.75% there was still less. After 48 hours, growth appeared in the tubes containing 2.0% to 2.75%; after three days in 3.0% and 3.25%; after eight days one of the set with 3.5% became turbid. Beyond this point no growth took place, 3.75% being sufficient to prevent growth.

The detailed results of this experiment are given in Table I.

TABLE I
The Effect of Sodium Chloride upon Growth in Bouillon

%NaCl	Days Before Growth Appeared														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
.50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
.75	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.00	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.75	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.00	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.25	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.50	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.75	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.00	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
3.25	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+
3.50	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+
3.75	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4.25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4.50	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4.75	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

22. GROWTH IN BOUILLON OVER CHLOROFORM.—To 10 c.c. of nutrient broth in test tubes, 5 c.c. of chloroform were added with aseptic precautions. Duplicate tubes thus prepared were inoculated with one loop of a 24-hour broth culture and held at 20° C. No growth took place in the tubes containing the chloroform after 60 days, while a check tube of plain broth gave abundant growth in 24 hours.

23. NITROGEN.—Peptone, asparagin, urea and ammonium tartrate have been used in different media as a source of nitrogen for the organism. Nitrogen was obtained very readily, as would be expected, from the first of these, as it occurred in nutrient broth, nutrient gelatin, nutrient agar and Dunham's solution. Good growth was also secured from the nitrogen of the asparagin present in Jordan's asparagin solution.

Jordan's Asparagin Solution

Redistilled water.....	1000 c.c.
Asparagin	2 grams
MgSO ₄	1 gram
K ₂ HPO ₄	1 gram

No growth was obtained from either the urea nitrogen in Sohngen's solution, or the ammonia nitrogen in the ammonium tartrate of Cohn's solution.

Sohngen's Solution

Tap water ..	500. c.c.
Urea	25. grams
K ₂ HPO ₄	.25 gram
Calcium malate	2.50 grams

24. BEST MEDIA FOR LONG-CONTINUED GROWTH.—Standard nutrient agar +10.

25. QUICK TESTS FOR DIFFERENTIAL PURPOSES.—Liquefaction of gelatin; coagulation and peptonization of plain milk; growth in Fermi's solution, absence of growth in Uschinsky's and Cohn's solutions; production of acid from dextrose, galactose and saccharose in sugar free broth; yellowish-green color produced in starch jelly.

III. PHYSICAL AND BIOCHEMICAL FEATURES

1. GAS PRODUCTION.—The power of the organism to produce gas from dextrose, galactose, saccharose, mannite, laevulose, maltose, inulin, lactose and glycerine was determined by adding 1% of the different fermentable substances to +10 sugar free bouillon. Two fermentation tubes of each were prepared, and after sterilization, they were inoculated with a 72-hour agar culture and placed in an incubator at 28° C. *No gas was formed in any case.* No growth occurred in the closed arm, and there was a sharp line of demarcation between the growth in the upper part of the U and the closed arm. After fourteen days the reaction of the different inoculated broths was tested with litmus, and it was found to be acid in the case of glucose, galactose and saccharose, and alkaline in the remainder.

2. AMMONIA PRODUCTION.—Nutrient bouillon containing 1% peptone and Jordan's asparagin solution with 0.2% asparagin were used in determining the power of the organism to produce ammonia. Duplicate 100 c.c. portions of each solution in 250 c.c. Erlenmeyer flasks were inoculated with two loops of a 24-hour broth culture and kept at 20° C. Uninoculated controls of each solution were carried along with these at the same time. After five days, both cultures and controls were analyzed for ammonia by distillation with mag-

nesia. The asparagin solution yielded considerably more ammonia than the nutrient bouillon. The results follow:

TABLE II
Ammonia Production

Nutrient solution	Milligrams N as NH ₃ in 100 c.c. culture after 5 days	Milligrams N as NH ₃ in 100 c.c. sterile culture solution	Milligrams N as NH ₃ produced by the micro- organisms
Nutrient bouillon...	15.1308 15.2709	9.03645	6.09435 6.23445
Asparagin solution..	27.4596 29.0707	0.9807	26.47890 28.09000

3. NITRATES IN NITRATE BROTH.—Nitrates are not reduced after five days either at 20° C. or 28° C.

4. INDOL PRODUCTION.—Neither indol nor the cholera red reaction is produced in Dunham's peptone solution after 10 days at 20° C.

5. ACID PRODUCTION FROM SUGARS AND GLYCERINE.—One hundred c.c. portions of +10 sugar free broth to which 1% of the different fermentable substances was added were inoculated with two loops of 24-hour broth culture and kept at 20° C. Five c.c. were taken from each flask with a sterile pipette daily and the reaction to phenolphthalein determined with n/10 NaOH or n/10 HCl. The titrations were made by adding the 5 c.c. of the sample to 45 c.c. of hot, freshly boiled distilled water, rather than by boiling the sample with the water. This was done in order to avoid the loss of any volatile acid that might be present. At the end of ten days there was a slight increase in the acidity of the broths containing saccharose, galactose and dextrose, from +10 to +13, altho in all probability this does not begin to represent the actual amount of acid produced, since considerable quantities must have been neutralized by the ammonia which we know is formed. The remaining broths were more alkaline at the end of the ten days than at the beginning. The detailed titrations are given in Table III.

TABLE III
Production of Acid from Sugars and Glycerine

250 c.c. Erlenmeyer flasks containing 100 c.c. sugar- free broth and 1 percent.	Reaction in degrees Fuller's scale after given number of days										
	0	1	2	3	4	5	6	7	8	9	10
Check	10	10	9	7	6	5	5	5	4	1	-2
Dextrose	10	10	10	10	9	9	9	9	11	13	13
Galactose	10	10	10	9	9	10	11	11	12	13	13
Saccharose	10	10	10	8	8	9	9	11	12	12	13
Mannite	10	8	8	8	6	5	4	4	4	2	-5
Laevulose	10	8	8	8	6	6	5	4	4	4	0
Maltose	10	8	8	6	6	5	5	5	3	-2	-2
Inulin	10	8	6	6	6	5	5	5	3	3	-3
Lactose	10	10	10	7	6	6	5	5	5	1	-4
Glycerine	10	8	8	8	5	5	4	4	4	4	4

6. HYDROGEN SULPHIDE PRODUCTION.—No hydrogen sulphide is produced. This was determined by means of both iron gelatin and nutrient broth.

In the former case, stab cultures were made in +10 nutrient gelatin which contained 0.5% of iron potassium tartrate. No blackening took place along the line of the stab as the result of the formation of iron sulphide.

In the second method of determination, tubes of nutrient broth were inoculated and held at 28° C. After 24 hours, strips of filter paper, moistened with lead acetate were suspended in the tubes above the fluid; these were remoistened each day for five days, but after fifteen days no blackening of the paper had taken place, due to the formation of lead sulphide.

7. TOLERATION OF ACIDS.—The toleration of acids is slight. We have determined this point with relation to citric, malic, oxalic and tartaric acids by adding different amounts of these to neutral nutrient bouillon in quantity sufficient to give the broth the acidities +15, +30 and +45. Three tubes of each acidity were prepared from each acid. They were inoculated with one loop of a 24-hour broth culture and subsequently kept at 28° C. In 24 hours, the +15 tubes of all the acids were uniformly turbid, but none of the others gave any growth. After twenty days the results remained unchanged.

Hydrochloric acid added to nutrient broth sufficient to give it a reaction of +27 inhibits growth.

8. TOLERATION OF ALKALIES.—So far as our studies go, the toleration of alkalies is even less than that of acids, altho this point has been determined for only one alkali, NaOH. Sodium hydroxide added to nutrient broth sufficient to give it a reaction -11 inhibits growth.

9. OPTIMUM REACTION.—The optimum reaction for growth in nutrient broth appears to be about +8. This was determined by adding to 20 c.c. portions of sterile broth, with aseptic precautions, sufficient normal NaOH or HCl as the case required, to give a series of broths ranging from +41° to -17° and differing from each other by 2 degrees. The contents of each flask were distributed into four sterile tubes by means of sterile pipettes, and without further sterilization, three of each set were inoculated with one loop of a 24-hour broth culture. The fourth tube of each set was kept uninoculated as a check against possible contamination, but in no case did any of these develop growth. The inoculated tubes were kept at 28° C.

After 24 hours, growth was present from +21 to -5, in 48 hours, it appeared in -7, and in 72 hours in +23, in 7 days in -9.

and in 12 days in +25. Beyond these points no growth took place, from which we may conclude that the limits of growth are from —9 on the alkaline side to +25 on the acid.

After 72 hours there was a marked difference in the degree of turbidity in the different tubes. From +5 to +11 the growth was best; from +13 to +19 there was a gradual decrease, and beyond +19 there was an abrupt falling off. Going in the other direction, there was a heavy growth in +3, but not as good as in +5; in +1 the turbidity was not as decided as in +3, and beyond this there was an abrupt drop.

From this, it appears that conditons for growth became very restricted beyond +19 on the one side and beyond +1 on the other, and altho growth was not entirely inhibited until +27 was reached in the one direction and —11 in the other, the optimum reaction seems to lie between +5 and +11, probably about +8.

10. VITALITY ON CULTURE MEDIA.—Long. By transplanting the culture once every two weeks on nutrient agar, no difficulty has been experienced in keeping it alive and active for ten months.

11. TEMPERATURE RELATIONS.—The thermal death point lies between 49° C. and 50° C. Ten c.c. portions of nutrient broth in thin-walled test tubes of uniform diameter (16 mm.) were inoculated with one loop of a 24-hour broth culture, and after allowing three to five minutes for thoro diffusion, four tubes for each temperature considered were plunged into water of the desired temperature up to the plugs. They were left there for exactly ten minutes and then immediately cooled in cold water. The water in the bath where the determinations were made was kept in constant motion, thus insuring a uniform temperature, by a horizontal paddle operated by a water motor. The effect upon growth was determined for each degree of temperature from 45° C. to 55° C. Good growth took place in all tubes heated up to and including 49° C., but none occurred beyond that.

Optimum temperature.—The best growth was obtained between 27° and 28° C. No growth takes place at 37½° C.; good growth occurs at 20°, but it is not as abundant as at 28°. At 7° C., sufficient growth results in 48 hours to define the line of inoculation on an agar streak, while in two weeks the growth is about the same as a 24-hour culture at 20° C., *i. e.* moderate.

12. EFFECT OF DRYING.—The organism is rather resistant to drying. Flamed cover-glasses were spread thinly with a suspension of a 24-hour agar culture in distilled water and allowed to dry in the air. They were afterwards placed in sterile, covered petri-dishes, and every 24 hours for a period of 22 days, two of

these were transferred to tubes of nutrient broth. There was considerable irregularity in the way these grew after different periods of drying, due presumably to the unevenness of the suspension. Up to 13 days drying, growth was fairly uniform, but none occurred again until the 18th day and then not until 21 days. No growth took place with the films dried for 22 days, but as none was dried longer than this, we can only say that 21 days dessication, under experimental conditions, was not sufficient to kill the organism.

13. PERCENT KILLED BY FREEZING.—(Salt and crushed ice.) The organism is relatively sensitive to freezing. From a 100 c.c. bouillon culture, 24 hours old, 10 c.c. were placed in each of four tubes of uniform diameter. These were packed in crushed ice and salt, frozen solid and kept frozen for 15, 30, 45, and 60 minutes respectively.

As soon as the specified time was up, each tube was thawed at once by placing it in cool water (four to five minutes required) and when entirely liquefied, dilution plates were made in +10 nutrient agar. Before freezing, dilution plates were made from the original culture as a check. Plates were kept at 20° C. and colonies counted after four days.

The original culture contained 19,800,000 bacteria per c.c. Eighty-five and 85/100 percent (8.85%) were killed by 15 minutes freezing; 93.03% by 30 minutes; 95.75% by 45 minutes, and 95.73% by 60 minutes. The number present after freezing 45 and 60 minutes is practically the same, altho the figures are slightly larger for the 60-minute tube; this is probably nothing more than an error due to dilution. The detailed results are given in Table IV.

TABLE IV
Sensitiveness to Freezing

Length of exposure to freezing temperature	Dilution	No. bacteria per c.c. after freezing	No. bacteria killed per c.c.	Percent killed
Check not frozen.	1-100,000	19,800,000	0	0
15 minutes	1-100,000	2,800,000	17,000,000	85.859
30 minutes	1-10,000	1,380,000	18,420,000	93.03
45 minutes	1-10,000	840,000	18,960,000	95.758
60 minutes	1-10,000	845,000	18,955,000	95.732

14. SUNLIGHT.—The organism is rather sensitive to the germicidal action of sunshine.

Flamed cover-glasses were spread thinly with a suspension of a 24-hour agar culture in distilled water and dried in the air. They were then exposed, germ side up, in covered petri-dishes to the bright sunshine. At the end of 15, 30, 45 minutes, 1, 1.5, 2,

2.5 and 3 hours, respectively, two cover-glasses were removed and dropped into tubes of sterile broth which were then inoculated at 28° C. Growth resulted from all exposures up to and including 2 hours, but none took place with the 2.5 and 3-hour slips. The experiments were made at Fort Collins, Colo., January 21, 1916, beginning at 10:55, a. m. and ending at 1.55, p. m.

Thinly sown agar plates, with one-half of the bottom covered with black paper were exposed to bright sunshine, bottom up, on a bed of crushed ice for 15 minutes. Exposure made February 24, 1916, 2:05 to 2:20, p. m. After exposure, the plates were held at 20° C. for four days and the colonies that developed counted. An average count of five plates showed that 33.2% of the organisms were killed by an exposure of fifteen minutes under the conditions of the experiment. The detailed results are given in Table V.

TABLE V
Effect of Sunlight

No. colonies exposed half petri-dish	on of	No. colonies covered half petri-dish	on of	Gain of covered over exposed	Percent killed by sunlight
21		30		9	30
22		38		16	42
28		46		18	39
24		32		8	25
25		36		11	30

15. RELATION TO OXYGEN.—The organism is an obligative aerobe. Duplicate inoculations upon agar slants and thickly sown agar plates were placed in Novy jars, and a stream of hydrogen was allowed to flow thru them for two hours, after which they were sealed. After fifteen days no growth had taken place on either slants or plates. When returned to the air, abundant growth followed on both, after 48 hours, showing that the organisms had not been killed, but merely prevented from developing because of lack of air.

Parallel stroke inoculations on agar plates were covered at intervals with flamed cover-glasses so as to exclude all air. No growth whatever occurred beneath the glasses, while abundant growth took place where the air was not excluded.

Further evidence of the aerobic nature of the organism is to be had in its failure to develop in the closed arm of the fermentation tubes.

16. FERMENTS.—A proteolytic enzyme, probably pepsin, and lab ferment are produced. Neither diastase nor invertase is formed.

17. CRYSTALS.—No crystals have been observed.

18. EFFECT OF GERMICIDES.—This has been reserved for a future publication.

IV. PATHOGENICITY

Pathogenic to field peas (*Pisum sativum* var. *arvense*) and garden peas (*Pisum sativum*). Not pathogenic to alfalfa (*Medicago sativa*), sweet clover—yellow—(*Melilotus officinalis*), crimson clover (*Trifolium incarnatum*), mammoth clover (*Trifolium pratense*, var. *perenne*), cow peas (*Vigna unguiculata*), and garden beans (*Phaseolus vulgaris*).

V. GROUP NUMBER

Ps. 211.2322033

FIELD EXPERIMENTS

In conjunction with Mr. F. H. Thomas, of Alamosa, we have endeavored to study the progress of the disease in the field under natural conditions. To this end, Mr. Thomas staked and marked 25 field pea plants, most of which were affected, on June 12, 1915, on the ranch of Mr. W. F. Uray between Monte Vista and Center.

These were observed at frequent intervals during the early summer, and changes in their condition from time to time were recorded as given in Table VI. The disease was most active from June 12 to June 17; on June 24 it had made but little further progress, and by July 1 it had practically disappeared.

Briefly, our study of these plants has brought out three points: First, badly affected shoots, where the infection is within a few nodes of the growing tip, are almost invariably killed outright; second, as a rule, when the original shoots are killed, new ones come up to replace them, except in very young plants; third, if the infection is confined to a few internodes next to the ground, the shoot outlives the disease and its growth is undisturbed.

TABLE VI

Observations on 25 Field Pea Plants on the Ranch of W. F. Uray, Center, Colorado, June 12 to June 24, 1915*

Plant No.	Date of Observation:		
	June 12, 1915	June 17, 1915.	June 24, 1915.
1.	West shoot badly infected; east shoot diseased at base.	West shoot diseased all the way to terminal tendril; still growing. East shoot diseased to second branch; growth** $\frac{1}{2}$ inch.	West plant ceased growing and throwing up new shoots 3 inches high. East plant, growth 2 in.
2.	Shoot to east—first 6 internodes badly diseased; trace on 8th and 10th; another shoot of same plant affected just on 1st internode; another shows trace.	East shoot dead; 2nd shoot diseased over 1st 7 internodes and traces on 8th and 9th; 3d just at base; 2nd and 3d growing.	New branches starting which have made 4-inch growth — old unchanged shoots.
3.	Slight infection at base.	Looks thrifty but infection solid over 5 internodes and on branch at 8th node; $\frac{3}{4}$ in. growth.	No advance in disease; whole vine growing—3 in.
4.	Slight infection at base of one shoot and others normal.	Infected shoot shows disease over 6 internodes, $\frac{1}{2}$ in. growth; normal shoot 3 in. growth; 1 dead shoot and 2 others affected over 4 internodes.	Four-inch growth on all shoots.
5.	East vine infected on 4 internodes; west not; just starting to branch.	East vine infected over 6 internodes; growth $1\frac{1}{4}$ in.; 2 new branches have developed; west shoot normal.	East plant blooming—growth 2 in.; normal.
6.	One shoot infected on first 3 internodes.	Diseased on first 6 internodes; growth 4 in.	No growth, although infection is not spreading.

*Observations of June 12 and 24 made by Mr. Thomas.

**The growth recorded refers to growth since last observation.

TABLE VI—Continued

Observations on 25 Field Pea Plants on the Ranch of W. F. Uray,
Center, Colorado, June 12 to June 24, 1915

Plant No.	Date of Observation:		
	June 12, 1915.	June 17, 1915.	June 24, 1915.
7.	Normal, no disease.	Stem and leaves both diseased on first 5 internodes. Many separate leaf infections; growth, 1— $\frac{3}{4}$ in.	Practically no changes.
8.	Not diseased, but inoculated by pricking into stem with knife infected from diseased plant. Second shoot pricked with knife before contamination.	Stem shows decided watery yellowish patches where inoculated. Check plant normal; growth 2 in.	Inoculated plant shows disease over 6 internodes; growth 2 in. Four new shoots coming up. Check plant normal; growth 4 in.
9.	Slightly infected, no marked discoloration.	Seven internodes involved, watery new shoots starting; $\frac{1}{2}$ in. growth.	Disease has not spread; growth 6 in. new shoots appeared, 1 in. long.
10.	Two shoots normal.	One shoot very badly involved, other normal, with $2\frac{1}{2}$ inch growth.	Diseased shoot practically dead; other branch normal; $2\frac{1}{2}$ in. growth.
11.	Entire clump of shoots infected.	All shoots sickly; $\frac{1}{4}$ in. growth.	Disease checked; growth 5 in.
12.	Normal.	Normal; growth 2 in.	Normal; growth 2 in.
13.	Badly infected up to 5th internode, inclusive.	Infected over 7 internodes; growth $\frac{3}{4}$ in. New shoots developing.	No growth; 1 new shoot dead and 3 others just starting.

TABLE VI—Continued

Observations on 25 Field Pea Plants on the Ranch of W. F. Uray,
Center, Colorado, June 12 to June 24, 1915

Plant No.	Date of Observation:		
	June 12, 1915.	June 17, 1915.	June 24, 1915.
14.	In reality 2 plants, red and white stem; disease just showing.	Red vine—3 shoots; 1 dead, 1 healthy, growing, 2 ½ in. 1 infected on 6th internode; no spread of disease; growth. White vine, traces just showing on petiole of bottom leaf; 1 in. growth.	Red vine—2 shoots growing, 2 ½ in. White plant — no internode; no spread of disease; 4-in. growth.
15.	Three shoots, slight traces.	One shoot dead, 2nd small, badly infected, 3d traces on 3d and 4th internode; branch at 4th node diseased; growth 3 in.	Second, dead; 3d, has grown 6 in., disease on 4th internode inactive.
16.	Normal.	Slight leaf infection on leaves at base; growth, 2 ½ in.	Disease has not spread; growth 2 in.
17.	Two diseased plants.	South plant diseased and watery to 9th node, new shoot starting, no growth. North plant, 4 branches: 1 dead, 1 badly affected, 2 normal; no growth. Red stem.	Original plants dead; 1 new shoot 8 in.
18.	Clump of small yellow shoots; badly diseased; nearly gone.	Practically dead; new shoots coming.	Original plants dead; 1 new shoot 8 in.
19.	West plant diseased; east normal.	West plant, petiole at 4th node affected, growth 1 in. East plant, slight infection on 1 branch, 3 healthy shoots; growth 2 in.	Disease has spread to entire plants, growth ½ in.

TABLE VI—Continued

Observations on 25 Field Pea Plants on the Ranch of W. F. Uray, Center, Colorado, June 12 to June 24, 1915

Plant No.	Date of Observation:		
	June 12, 1915.	June 17, 1915.	June 24, 1915.
20.	Just showing disease below first branch.	One entire shoot dead and 1 healthy; growth 2 in.	No growth on old shoots; new shoots growing rapidly, 6 in.
21.	Slight infection at base.	One shoot frosted, 1 shows trace of disease at 2nd internode, and 1 infected at growing tip. Second shoot has grown 2 in.	Growth 1½ in. Plants same.
22.	White vine diseased at base; red vine normal.	Disease up to 3d node on white vine, and side branch involved. Red vine normal.	Disease to 5th node on white vine, growth 6 in. Red vine growing, 6 in.
23.	Disease beginning to show on lower leaves.	Three shoots: 1 dead, 1 diseased to 5th node, and 1 healthy.	Diseased branch has grown 1 in.; healthy shoot, 4 in.
24.	Normal.	Leaf infections common and 1 shoot affected to the 8th node. Normal stem has grown 2-¾ in.	Little change, practically no growth on infected stems.
25.	Normal.	Three shoots: 1 dead, 1 diseased, over 4 internodes, and 1 normal.	Diseased shoot has grown 4 inches; normal, 6 in.

Observations, July 1, 1915

Mr. Thomas visited this field on July 1 and found all remaining plants making good growth. The vines were badly matted around the stakes so that it was impossible to identify the particular shoots observed before. There was some evidence of whole plants having been killed, but in the majority of cases, new shoots replaced badly infected ones while stems with the disease on lower leaves and internodes either branched below the infection, or, in many cases, continued to grow in advance of the disease.

In connection with extensive variety tests which Mr. Thomas was conducting on field peas for forage purposes at La Jara, we have had an excellent opportunity to observe disease resistance to the bacterial blight. Out of the 48 varieties which we examined, there were 8 desirable varieties from a forage standpoint that showed any appreciable degree of resistance. Almost without exception, the best forage peas were the worst affected by the disease. The results of our observations on disease resistance are given in Table VII. The peas were planted May 3, 1915, and observed June 17, 1915.

Brief descriptions of the forage qualities of the different peas follow:

OBSERVATIONS MADE AT LA JARA, COLORADO, AT TIME OF HARVEST, BY E. H. THOMAS, COUNTY AGENT

All varieties planted under same conditions and at same time; four fifty-foot rows of each. Planted May 3, 1915. Seed furnished by Bureau of Forage Crop Investigations and by experiment stations of Idaho, Washington, Oregon, Minnesota and Ontario. Some varieties were also furnished by the W. A. Davis Seed Company, and the Allen Seed Company. Some seed was purchased from points in the north and west.

NO. 11087.—EUGENE.—Produced slender short vine at La Jara. Yield of forage fairly heavy, with many well-filled pods. Should be excellent pea seed for heavy land, fairly early maturing.

NO. 16130.—GOLDEN VINE.—Produced a tall, medium-sized vine; fairly good quantity of forage, but with few pods and they were poorly matured. Might make good if planted early in light soil.

NO. 16436.—GREY WINTER.—Produced medium length, small-sized vine. Produced good quantity forage. Was extremely well podded but rather immature. Believe that peas should be sown probably on heavy or medium heavy soil late in fall or very early spring.

NO. 18455.—SHANGHAI.—Produced medium length, slender vine. Yielded fair amount of forage. Many medium-sized pods containing rather hard, medium-sized peas which shatter rather easily. Would no doubt make good forage or grazing pea on fairly strong land. Medium late.

NO. 19788.—POTTER.—Produced long coarse vine. Good amount forage. Yielded fair number pods, but poorly matured. Would probably make excellent forage pea on medium or heavy soil. Should be sown very early in spring.

NO. 20467.—SOLO.—Grown on medium, heavy land, produced long, medium-sized vine, yielding very good quantity of forage. Vine is extremely well podded thruout entire length; peas are rather soft. This variety should be excellent forage pea on heavy land and seed on lighter soils.

NO. 21288.—BANGALIA.—Slender, short vine, produced rather moderate quantity of forage when grown on medium, heavy soil. Produced a great many small-sized pods; rather early, drouth-resisting, should be great seed producer on heavy soils (or medium). Land should be fairly clean.

NO. 21289.—DESI.—Great number small, well-filled pods on short vine. Yield of forage light. Should make great grain production on heavy or medium heavy soils. *Do not* shatter easily.

NO. 21290.—KABILYA.—Produces medium vine. Good amount forage. Many pods. Excellent forage pea for heavy land; probably good seed pea on lighter soils. Should be planted early in spring.

NO. 21709.—AMORITI.—Medium length small-sized vine; fair yield forage. Many pods. Should be good pea for all classes of land. Seed and forage for heavy soils, also for medium heavy, and seed for light soils. Matures in good season, but should be planted rather early.

NO. 22007.—ALEXANDER.—Only medium yield of vine and seed. Does not seem to be anything extraordinary. Not enough pods for heavy land, nor enough vine for light land. Only fairly good yielder.

NO. 22036.—AGNES.—Long, coarse vine; fairly heavy amount of forage, but does not produce many pods, and is very late, should be planted very early in spring. Might make good forage pea for light land.

NO. 22037.—ARCHER.—Has long, coarse vine, good yield of forage, fair number of pods. More seed than 22036, but nothing extra; might make good forage pea on lighter soils.

NO. 22041.—GREGORY.—Same general type as 22037, but with fewer pods. Rank vine. Might make good forage pea for light soils. Should be planted very *early*.

NO. 22044.—PARAGON 12. Long, medium-sized stem; heavy yield of forage; medium number of pods. Might be good pea on lighter soils. Should be planted *very early*.

NO. 22078.—KILLARNEY.—Rather long, coarse stem, producing good quantity of forage. This variety is extremely well podded, compares rather favorably with 22043—Nelson. Should

be sown on light land for grain and early on medium or heavy land for either seed or forage. Might make good forage on light land.

NO. 22638.—CLAMART.—Vine tall or long and coarse; yields great quantity of forage. Produces very few pods. Would probably be good pea for lighter grades of soil. Should be planted early, so as to insure maturing of pods.

NO. 22639.—WHITE SCIMITAR.—Vines of medium length; slender stems. Yielding fair amount of forage. An excellent producer, a great many pods from $1\frac{1}{2}$ to 2 inches in length. Should be planted quite early, as this variety needs all the time possible to mature. A very good pea; would probably give best results from medium light land.

NO. 23290.—CHANG.—Medium length vine, coarse stem, yields good quantity forage. Many pods on upper or top half of vines, lower portion not so well podded. Would probably do better, pod more evenly if grown on light, sandy soil.

NO. 23848.—Vine long and fairly coarse. Fairly heavy yield of forage. Only moderate number of pods, but fair forage specimen; should be sown early.

NO. 23850.—LIMA.—Vines grow long and moderately coarse. Yields good amount of forage. Good many pods; very good pea, but should be planted early on light or medium light soils to secure best results for seed.

NO. 24314.—FRAILE.—Medium vine and stem; yields good quantity forage. Fairly well podded. Should be planted early to mature in good shape. Does not yield a great amount of either vine or grain.

NO. 25439.—OPENSHAW.—Vine of medium length, stem coarse. Good yield of forage. Great many pods. A good pea for almost any kind of land; forage and seed on heavy land, and seed on lighter grades. Plant fairly early.

NO. 25680.—BROWN ABYSSINIAN.—Medium vine and stem; fair yield of forage. Good yielder; many pods. *Does not* shatter easily. Should be good pea for harvesting; would grow in all grades of soil; earlier than all but a few varieties.

NO. 29368.—BLUE PRUSSIAN.—Vines medium, stems medium, yield of forage fair. Great many pods; would probably do well in clean, medium heavy land.

NO. 29371.—CHURCH.—Vines tall; medium stems; very good yield of forage. Many pods, very good pea. Would probably seed heavily on light soils. Should be planted rather early.

WHITE MARROWFAT.—Stems coarse, vines of medium length, yield of forage good. Great many good-sized pods. Should be planted early to insure maturing.

CHANCELLOR.—Medium vine and stem; good yield of forage; many well developed pods on vines. Good pea for heavier soils as well as excellent for seed on light soils.

TOM LEXINGTON.—Not much vine; yield of forage only fairly good. Quite a number of good-sized pods. A beautiful pea. Should be sown on good grade of clean ground.

McKAY.—Very rank vine, yields much forage, very few pods. Would probably do better if planted early on higher lighter soil.

PROLIFIC.—Vines short; stem medium. Yield of forage rather light. A great many well-developed pods; peas are rather hard, might be grazed, as they shatter rather easily; would probably grow best on medium soil, but ought to be good peas on any clean ground.

ADVANCER.—Short slender vine, yield of forage rather light. Many fairly well-developed pods. Might do well on high medium heavy ground.

CARLTON.—Vines long and stem medium; fair yield of forage. Vine fairly well podded on upper position, lower 24 inches decayed at time of harvest. Might plant rather thin on higher land. Plant early.

ARTHUR SELDEN.—Vine very coarse and short. Good yield of forage. Good number fair-sized pods. Should be planted very early (when compared with early varieties).

PICKTON.—Vine rank and coarse, heavy yield forage. Good many fair-sized pods. Should plant early on light or medium soils.

EARLY FORAGE.—Short, sturdy vine. Pods mature even earlier than Horsford, and is heavier podded. Peas are soft, wrinkled and sweet; ought to be excellent for early grazing, yield of forage good, considering size of vine. Believe that this variety should be sown rather thick; should do well on heavy land.

TABLE VII

Disease Resistance—Variety Tests at La Jara, Colorado, Conducted by
Mr. E. H. Thomas. Season 1915—Observations made June 17, 1915

Variety No.	Variety Name.	Prevalence of Disease.	Quality as Forage Pea.
11097	Eugene	Bad	Good
16130	Golden Vine	Very bad	Very good
16436	Gray Winter	Bad	Poor
18455*	Shanghai	Slight	Good
19788	Potter	Bad	Poor
20467*	Solo	Slight	Good
21288	Bangalia	Bad	Very good
21289	Desi	Moderate	Poor
21290*	Kabilya	Very slight	Good
21709	Amoriti	Bad	Good
22007	Alexander	Very slight	Poor
22036	Agnes	Bad	Fair
22037	Archer	Moderate	Poor
22041	Gregory	Considerable	Poor
22044	Paragon 12	Moderate	Good but late
22078*	Killarney	Slight	Very good
22638	Clamart	Very bad	Poor
22639	White Scimitar	Very bad	Poor
23290*	Chang	Slight	Very good
23848		Very bad	Good
23850	Lima	Slight	Fair
24314	Fraile	Very bad	Poor
25439	Openshaw	Very bad	Very good
25680	Br. Abyssinian	Slight	Fair
29368	Blue Prussian	Bad	Good
29371	Church	Bad	Good
	White Marrowfat	Considerable	Fair
	Chancellor	Moderate	Fair
	Tom Lexington	Very bad	Very good
	McKay	Bad	Good
	Prolific	Bad	Good
	Advancer	Very bad	Good
	Carlton	Bad	Poor
	Arthur Selden	Very bad	Very good
	Pickton	Bad	Fair
	Early Forage	Very bad	Very good
	Braden	Moderate	Good
	White Mexican	Very bad	Good
	Paragon	Very bad	Good, but late
	Late Forage	Bad	Very good
	Minn. 62	Bad	Good
26819		Very bad	Very good
31073*		Slight	Good
32003		Very slight	Fair
32061		Slight	Fair
32065*		Very slight	Good
32104*		Slight	Good
32123		Very slight	Fair

The promising varieties are marked with an asterisk ().

METHOD OF INFECTION

Both our laboratory experiments and our field observations indicate that the causal organism enters the plants either thru the stomata or thru wounds produced by mechanical injuries.

In looking into the history of the trouble last spring we found one striking case where the outbreak unquestionably followed cultivation with a spike-toothed harrow at a time when the wind was carrying quantities of soil. The custom of harrowing to break the crust and to conserve moisture is a dangerous practice, because it wounds the tender plants and opens the way for soil infection. This was brought to our attention in a very graphic manner in a field where a large number of varieties had been planted for experimental purposes. A spike-toothed harrow had been dragged across the lower part of one of these rows and the upper end left untouched; all of the varieties blighted more or less in that portion of the row that was harrowed, while in the unharrowed part several of the same varieties exhibited marked resistance.

Two other instances were noted where infection followed closely upon a severe sand-storm which bruised the vines and literally injected the tissue with germ-laden soil particles. In fact, soil-laden wind alone, where it blows as violently and persistently as it does in Colorado in the springtime, is adequate explanation for all the infection that might ever occur in our State.

CONTROL MEASURES

1. Resistant varieties offer the only satisfactory remedy for the blight, and in the next two years we shall endeavor to obtain strains that are disease-resistant and, at the same time, desirable types both for forage and canning purposes.

2. In the meantime, when practicable, we would suggest planting from ten days to two weeks later than is the local custom.

3. So far as possible, avoid mechanical injury to the plants by harrowing or by other similar practices.

4. Avoid planting peas on the same land that was in peas the previous year, or where the disease has been present.

5. While we have had no opportunity as yet of determining whether the blight organism can pass thru the digestive tract of animals, fed with diseased vines, unharmed, and thus infect the manure, it may not be out of place to suggest the possibility of this at this time, and if we should have occasion to use any such manure on our gardens or in our fields, to remember that we may be sowing the seed of endless woe. Until we know definitely that

such fertilizer is free from this objection, its use should be regarded as a doubtful practice.

SUMMARY

1. The bacterial stem blight of field and garden peas occurs generally over the San Luis Valley and Northern Colorado, and to a limited extent in Nebraska, South Dakota and Utah.

2. It is characterized by the watery, olive-green to olive-brown color of the stems, and by the yellowish, bruised and watery-appearing stipules and leaflets.

3. A severe outbreak when the plants are young may reduce the stand one-third.

4. The blight is caused by *Pseudomonas pisi*, n. sp., which enters the tissue thru the stomata and thru wounds produced by mechanical injury.

5. Resistant varieties offer the only satisfactory remedy for the trouble, altho later planting may reduce the amount of the injury somewhat.

ACKNOWLEDGMENT

I am very greatly indebted to Professor B. O. Longyear for the preparation of the colored plate which appears in this bulletin, and to Mr. E. H. Thomas, of Alamosa, Colorado, for the many courtesies extended to me while studying the disease in the San Luis Valley, and for much of the field data presented herewith.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1.—Pea leaf sprayed with a suspension of *Ps. pisi*, showing stomatal infection six days after inoculation.

FIG. 2.—Gelatin stab culture of *Ps. pisi*, kept at 20° C. and photographed after three days. Broad funnel-shaped liquefaction at surface, and slight growth along the line of the stab.

FIG. 3.—Gelatin stab culture of *Ps. pisi*, kept at 20° C. and photographed after eight days. Stratiform liquefaction at the surface, and slight growth along the line of the stab.

PLATE II.

FIG. 1.—Photomicrograph of *Ps. pisi* stained with carbol fuchsin, 24-hour agar culture. $\times 1000$.



PLATE I



1.



2.



3.



4.



5.

PLATE II



Fig. 1



Fig. 2

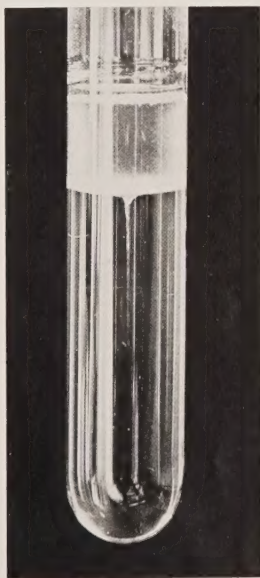


Fig. 3

FIG. 2.—Chains of *Ps. pisi* from 24-hour agar culture. Stained with carbol fuchsin. $\times 1000$.

FIG. 3.—Agar plate culture of *Ps. pisi*, showing surface and deep colonies. Five days old. Actual size.

FIG. 4.—Surface colony from agar plate. Five days old. $\times 100$.

FIG. 5.—Deep colonies of *Ps. pisi* from agar plate. Five days old. $\times 100$.

COLORED PLATE.

LEFT-HAND FIGURE.—Characteristic yellow, olive green, watery stipules and stem; recent infection.

MIDDLE FIGURE.—Diseased pea vine with olive green, watery stem and healthy shoot coming from below diseased portion. Infection active.

RIGHT-HAND FIGURE.—Pea vine in advanced stage of the disease.

